Pseudohyperkalemia

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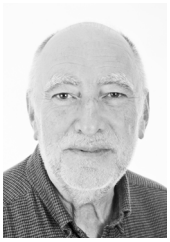

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In health plasma/serum potassium (K^+) is maintained within the approximate reference range of 3.5 to 5.2 mmol/L, with serum values being slightly higher (~ 0.4 mmol/L) than those of plasma because the process of blood clotting, essential to recovery of serum samples, is associated with release of potassium from activated platelets.

Hyperkalemia (increased potassium) is diagnosed if patient result exceeds the upper limit of the local reference (normal) range. Severe hyperkalemia, usually defined as serum/plasma K^+ >6.5-7.0 mmol/L is associated with risk of potentially fatal cardiac arrhythmia and warrants emergency clinical intervention. Probably, the two most common causes of hyperkalemia are chronic kidney disease (CKD) and certain prescribed drugs, but there are many others and, in particular cases, the cause is often multifactorial.

Determining cause(s) is vital in assessment of a patient presenting with unexplained hyperkalemia. This determination should include consideration of the possibility that the potassium result is falsely (spuriously) raised, and that this is, in fact, a case of pseudohyperkalemia, the subject of this article.

The terms pseudohyperkalemia, reverse pseudohyperkalemia, familial pseudohyperkalemia and seasonal pseudohyperkalemia will be explained here, but the main focus of the article will be two broad causal aspects of pseudohyperkalemia: poor technique or practice during collection and preanalytic processing of samples; and pathological conditions that predispose to pseudohyperkalemia. The article begins with a brief discussion of potassium distribution in the body, which is helpful for understanding the mechanism(s) responsible for pseudohyperkalemia.

Background physiology

Potassium (K^+) is the most abundant cation in the body and nearly all (~98 %) of total body K^+ (which amounts to approximately 3,500 mmol or 139 g) is contained within tissue cells [1]. Skeletal muscle is the most abundant tissue type, so skeletal muscle represents the largest reservoir of potassium in the body. In common with the cells of all other tissue types, the intracellular fluid (ICF) potassium concentration of skeletal muscle cells is of the order of 100-150 mmol/L. The remaining ~2 % of total body potassium that is not contained within tissue cells is present in the extracellular fluid (ECF) at a concentration of just 4-5 mmol/L [1].

Since cell membranes are permeable to ionic species such as K^+, maintenance of the large concentration gradient (ICF K^+ 100-150 mmol/L vs ECF K^+ 4-5 mmol/L) across the cell membrane, essential for many cell functions, depends on the energy-consuming sodium-potassium pump (Na^+-K^+-ATPase) present in the membrane of all cell types. This drives Na^+ out of cells and K^+ into cells, so whereas potassium is a predominantly intracellular cation, sodium is a predominantly extracellular cation [1].

These physiological aspects of potassium distribution apply equally to blood, a connective tissue. Blood plasma, which is that relatively small (approximate 25 %) portion of the total body ECF compartment that is contained within the vasculature, has a potassium concentration close to 5 mmol/L, and the three cell types that circulate suspended in blood plasma – erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets) – have an ICF potassium concentration close to 100 mmol/L.

Since erythrocyte numbers far outstrip those of the other two blood cell types, erythrocytes represent by far the largest reservoir of K^+ in blood. As in other tissues, maintenance of the large differential between plasma and blood cell potassium concentration depends on the sodium-potassium pump (Na^+-K^+-ATPase) present in the membrane of blood cells. The glycolytic pathway that ensures generation of energy-rich ATP from glucose is essential to the continuing function of the pump, so glucose can be considered the primary "fuel" that drives the pump.

The high concentration of potassium within blood cells relative to plasma concentration determines that a small release of potassium from blood cells can significantly increase plasma potassium concentration. For this reason, accurate determination of in vivo plasma potassium concentration demands that the process of blood collection and handling preserves the physical and functional integrity of blood cell membranes until plasma or serum is separated from cells.

As we will see, all of the many potential causes of pseudohyperkalemia can be explained in terms of *in vitro* reduction in blood cell (membrane) integrity or function and consequent *in vitro* release of potassium from blood cells to plasma or serum.

Definitions: pseudohyperkalemia, reverse pseudohyperkalemia and familial pseudohyperkalemia

Pseudohyperkalemia (alternative names: spurious hyperkalemia, factitious hyperkalemia, and artefactual hyperkalemia) is falsely raised serum or plasma potassium concentration. That is, the measured (*in vitro*) value is above the upper limit of the local reference range when the actual (*in vivo*) potassium concentration is within the local reference range [2]. The mechanisms that give rise to pseudohyperkalemia can also result in patients who have a reduced potassium (*in vivo* hypokalemia) being falsely identified as having a normal potassium (*in vitro* normokalemia). This is sometimes referred to as "masked hypokalemia" [3].

Pseudohyperkalemia is one of the most common testing errors that occur in the clinical laboratory [4]

and obviously should provoke no treatment. Failure to identify pseudohyperkalemia and treating as if the high result were valid could result in severe – potentially life-threatening – hypokalemia. Unidentified "masked hypokalemia", on the other hand, prevents patients receiving the potassium supplement therapy they may well need.

Pseudohyperkalemia was first described in the 1950s among patients with marked increase in platelets (thrombocytosis) [5]. Affected patients had increased serum potassium but much lower (normal) plasma potassium. As a result of this early observation, pseudohyperkalemia has been defined as marked elevation of serum potassium (>0.4 mmol/L) compared to plasma potassium [6].

This narrow definition is flawed in the sense that it implies that pseudohyperkalemia is confined to serum samples, and that a policy of using only plasma samples for potassium estimation would eliminate the problem of pseudohyperkalemia. This is clearly not the case; a number of the mechanisms, outlined below, that can give rise to pseudohyperkalemia affect serum and plasma samples equally.

The term "reverse pseudohyperkalemia" was coined fairly recently to describe rare instances when plasma potassium is falsely raised but concurrently collected serum potassium is normal [6, 7]. The cases reported thus far are confined to patients with hematological malignancy and associated extreme increase in leukocytes numbers. This is discussed in a little more detail below.

Familial pseudohyperkalemia, sometimes called leaky cell syndrome [8], describes a particular and rare inherited presentation of pseudohyperkalemia; it is discussed in a little more detail below.

Causes of pseudohyperkalemia (1): Poor technique or practice during collection and preanalytic processing of blood samples

The many ways in which error during sample collection and handling can result in pseudohyperkalemia can be conveniently addressed under the following four mechanistic headings:

- *In vitro* hemolysis [10]
- Fist clenching during phlebotomy [11, 12]
- Undue delay in processing blood samples [13]
- Inappropriate storage temperature of blood samples [13]
- Potassium contamination of blood samples [14, 15]

In vitro hemolysis is the most common cause of pseudohyperkalemia. Hemolysis is the rupture (lysis) of erythrocytes with consequent release of their contents, including potassium and hemoglobin, to plasma [10]. *In vivo* (intravascular or extravascular) hemolysis is a rare pathological feature of a number of diverse conditions and accounts for <2 % of all cases of hemolysis [10]. Any increase in plasma (or serum) potassium resulting from *in vivo* hemolysis is true hyperkalemia. By contrast, *in vitro* hemolysis is a process that only occurs in blood removed from the body and is due to mechanical disruption of erythrocytes induced by the process of blood collection and handling.

Release of hemoglobin from erythrocytes during in vitro (and *in vivo*) hemolysis causes a color change in plasma and serum. Plasma/serum is normally light straw colored but the presence of significantly increased amounts of hemoglobin turns it pinkish through to deep red, depending on the serum/plasma concentration of hemoglobin (severity of hemolysis). Visual inspection of plasma/serum thus provides the means for identifying hemolyzed samples and, thereby, those at risk of resulting pseudohyperkalemia.

Many modern analyzers now routinely quantify

the concentration of hemoglobin in plasma and serum samples and report a so-called hemolysis index (HI) [10]. This is a more sensitive and reliable means of flagging samples affected by hemolysis than visual inspection. So long as *in vivo* hemolysis can be excluded, the combination of increased HI and increased plasma or serum potassium is highly suggestive of pseudohyperkalemia.

Review articles and case study reports [3, 10, 16-18] have outlined various aspects of the preanalytic (blood collection and handling) process that can deleteriously impact on the integrity of erythrocytes and cause in vitro hemolysis. They include:

- Use of narrow-gauge needles
- Use of syringe and needle rather than evacuated tube collection systems
- Sampling blood via IV catheter
- Non-standard (i.e. other than antecubital fossa) venipuncture site
- Prolonged use of tourniquet
- Vigorous shaking of samples after collection
- Transportation of samples via some pneumatic tube transport systems (PTS)
- Long-lasting /excessive centrifugation

Fist clenching during phlebotomy is a practice widely used by phlebotomists; patients are asked to clench their fist in order to make veins more prominent. A number of studies [11, 12, 19] have demonstrated that this practice is associated with risk of pseudohyperkalemia. It is widely supposed that the mechanism of the pseudohyperkalemia is release of potassium from forearm muscle cells to surrounding interstitial fluid consequent on repeated or prolonged fist clenching.

Hemolysis may also be a contributory factor. Fist clenching should be avoided when collecting blood for potassium estimation [19]. Seimiya *et al* report reduced incidence of pseudohyperkalemia after implementing a policy of avoiding fist clenching during phlebotomy [20].

Undue delay in processing blood samples is a potential cause of pseudohyperkalemia because the maintenance of *in vivo* potassium concentration depends on continuing (*in vitro*) activity of the sodium-potassium pump (Na^+-K^+-ATPase). When blood is removed from the body, it cools towards room temperature. This progressive cooling reduces activity of the glucose-metabolizing (glycolytic) enzymes that generate ATP necessary for sodium-potassium pump function.

As a consequence of the fall in temperature, therefore, activity of the pump decreases with consequent efflux of potassium from blood cells to plasma/serum. Eventually, all the glucose in the blood at the time of sampling is consumed and ATP can no longer be generated. At this point the pump ”fails” and potassium diffuses passively out of blood cells.

These considerations determine that there is an increasing risk of pseudohyperkalemia the longer plasma or serum remains in contact with blood cells after blood is sampled [13]. To eliminate this risk, plasma or serum should be separated from cells by centrifugation as soon as possible after blood is collected, and certainly within 3-4 hours of collection. One authority [21] recommends separation of serum from cells within 2 hours of blood collection. There is an inevitable delay in recovering serum samples because of the time-dependent (0.5 hour) process of clot retraction. In this sense serum samples are potentially more prone to pseudohyperkalemia due to time delay than plasma samples.

Inappropriate storage temperature of blood samples during the time between collection and separation of serum or plasma from blood cells is a potential contributory cause of pseudohyperkalemia. Freezing whole-blood samples, for example, causes massive in vitro hemolysis and thereby severe pseudohyperkalemia. As already discussed, less severe cooling of blood is associated with reduced activity of the sodium-potassium pump

and thereby *in vitro* efflux of potassium from blood cells to plasma or serum.

The tendency to pseudohyperkalemia is greater for samples stored in a fridge at 4 °C than for samples stored at ambient room temperature ~20 °C. So it is recommended that blood be stored at ambient temperature (15-25 °C) during the time between blood collection and separation of serum or plasma [4, 22]. The particular problem of pseudohyperkalemia due to delay in processing samples sent to laboratories from far-flung General Practice (GP) surgeries, was examined by Sinclair D *et al* [23].

They demonstrated a phenomenon they called "seasonal pseudohyperkalemia". Pseudohyperkalemia was more frequent in winter months when ambient transport temperature was low (3-12 °C) than in summer months, when ambient transport temperature was higher (18-25 °C). A more recent study [24] confirmed that installing a centrifuge in GP surgeries, allowing serum or plasma to be separated from cells before transport, eliminates "seasonal pseudohyperkalemia".

Potassium contamination of blood samples is a potential cause of pseudohyperkalemia. The most common contaminant is the potassium salt of ethylenediaminetetraaceticacid (K^+EDTA) [14, 15], an anticoagulant additive present in tubes used to collect blood destined for hematological testing (FBC, CBC).

Three modes of K^+EDTA contamination have been proposed; all represent poor phlebotomy technique. The first involves simply decanting blood from K^+EDTA tube to the tube used to collect blood for potassium testing. The second is incorrectly drawing blood into a K^+EDTA-containing bottle before drawing blood into sample tube for potassium estimation.

This so-called "order of draw" error can result in backflow of K^+EDTA-contaminated blood into the vacutainer collection system and subsequent transfer to the tube used to collect blood for potassium estimation. However, recent study [25, 26] has challenged the view that incorrect order of draw when using a closed vacutainer system can lead to K^+EDTA contamination and pseudohyperkalemia.

The third mode of K^+EDTA contamination occurs when syringe and needle rather than vacutainer collection systems are used to collect blood samples. Syringe needle tips may become K^+EDTA-contaminated during transfer of blood from syringe to K^+EDTA-containing tube. This needle contamination can "infect" all subsequent blood collection tubes including those used for potassium estimation. It is important to transfer blood from syringe to tubes for potassium estimation before transferring blood to K^+EDTA-containing tubes.

Quite apart from K^+EDTA contamination, blood samples may become contaminated with potassium if blood is sampled from the same arm that is being used to administer potassium-containing IV infusion.

Causes of pseudohyperkalemia (2): Patient conditions that predispose to pseudohyperkalemia

The vast majority of pseudohyperkalemia cases result from one or more of the many preanalytical errors outlined above. Some cases of pseudohyperkalemia, however, occur despite best practice in collection and preanalytic handling of specimens. Cause, in this minority of cases, lies in the patient being tested. Here we consider the three pathological conditions that predispose to pseudohyperkalemia. They are:

- Inherited defects in erythrocyte membrane structure
- Marked increase in platelet count (thrombocytosis)
- Marked increase in white cell count (leukocytosis)

Two inherited defects in erythrocyte membrane structure predispose to pseudohyperkalemia; they are familial pseudohyperkalemia (FP) and dehydrated hereditary stomatocytosis (DHS).

FP is a condition that results from inheritance of mutation in the gene (ABCB6) that codes for the erythrocyte membrane protein, ABCB6 [27]. Mode of inheritance is autosomal dominant and several mutations have been described, one of which is quite common (present in 0.3 % of blood donors tested [27]). The genetic anomaly that defines FP causes increased in vitro leak of potassium from erythrocytes to plasma/serum when blood is exposed (*ex vivo*) to temperatures below normal body temperature (37 °C) [9]. Affected individuals are asymptomatic and suffer no known deleterious effects as a result of FP, apart from this risk of pseudohyperkalemia.

DHS is an inherited defect (mutation) in the gene (PIEZO1) that codes for the erythrocyte membrane protein, PIEZ01 [27]. Like FP, it is inherited as an autosomal dominant trait. Unlike FP, however, it is not necessarily a benign condition. Phenotype expression of the inherited gene defect is variable but most DHS-affected patients show well-compensated hemolytic anemia. A proportion of DHS-affected patients exhibit the same increased temperature-dependent in vitro leakage of potassium from erythrocytes to plasma/serum, and associated pseudohyperkalemia evident in those with FP.

It is vital for accurate determination of potassium status of those with FP and DHS that blood is collected into anticoagulant, and plasma is separated from cells immediately blood is collected. If there is unavoidable delay, then blood must be maintained at 37 °C between the time of collection and centrifugation to prevent in vitro leakage of potassium from erythrocytes and consequent pseudohyperkalemia.

Point-of-care biochemical analyzers (including blood gas analyzers) often have the capacity for measurement of potassium on whole-blood samples immediately the sample is collected. This mode of analysis is also well suited for accurate assessment of in vivo potassium status among these patients.

Marked increase in platelet count (thrombocytosis) was the first identified cause of pseudohyperkalemia [5]. It is due to increased in vitro release of potassium from activated platelets during the process of clotting and is therefore only a problem if serum is used to measure potassium. Plasma potassium is unaffected by marked increase in platelet numbers [28, 29].

Thus, in the context of thrombocytosis, pseudohyperkalemia can justifiably be defined as marked elevation of serum potassium (>0.4 mmol/L) compared to plasma potassium. Ranjitkar *et al* [29] demonstrated a positive linear relationship between platelet count and the extent to which serum potassium is spuriously increased. Their data suggests that serum potassium is falsely increased by 0.05 mmol/L for every 100 x 109/L increase in platelet count.

They and others [30] suggest a threshold platelet count of >500x109/L should be used to indicate high risk of pseudohyperkalemia (i.e. serum potassium being falsely increased). All those with platelet count in excess of 500 x 109/L should have their potassium status assessed using plasma recovered from an anticoagulated blood sample or, alternatively, an anticoagulated whole-blood sample.

Extreme increase in white cell count (extreme leukocytosis) associated with hematological malignant disease can occasionally cause pseudohyperkalemia or reverse pseudohyperkalemia, most notably in patients with chronic lymphocytic leukemia (CLL) and very high white cells counts (>150 x 109/L) [31, 32]. Both serum and plasma samples can be affected though recent evidence

[29] suggests that the impact of high white cell count on potassium is greater for plasma samples than serum samples.

Such a view is supported by Hong-Kee *et al* [33] who studied evident pseudohyperkalemia in four patients with CLL and very high white cell counts (all >120 x 109/L). In all cases plasma potassium was raised (in the range of 5.6 - 7.8 mmol/L) but serum potassium was normal (in the range of 3.1 - 4.8 mmol/L). These were all cases of reverse pseudohyperkalemia (plasma potassium raised, serum potassium normal).

The mechanism of pseudohyperkalemia in the context of extreme leukocytosis is presumed to be in vitro white cell lysis and consequent efflux of potassium from white cells to plasma/serum, but precise cause of lysis is uncertain. Leukemic white cells are often fragile and therefore more prone to lysis; this may well be significant. The anticoagulant heparin used to prepare plasma samples has been shown to promote white cell lysis [29] and this may in part explain the preponderance of pseudohyperkalemia due to extreme leukocytosis in plasma rather than serum samples.

There have been a number of case study reports [34 - 37] implicating pneumatic tube transport systems (PTS) as the primary or contributory cause of extreme leukocytosis-related pseudohyperkalemia. It is supposed that fragile leukemic white cells are particularly susceptible to lysis consequent on mechanical trauma they suffer during transport via PTS. Authors of these case study reports caution that blood destined for potassium analysis from patients with extreme leukocytosis should not be transported via PTS.

Ranjitkar *et al* [29] demonstrated a positive linear relationship between white blood count and the extent to which plasma potassium is spuriously increased. Their data suggests that plasma potassium is spuriously increased by 0.6 mmol/L for every 100 x 109/L increase in white blood count. They recommend that a threshold white blood count of >50 x 109/L be used to indicate high risk of pseudohyperkalemia. If pseudohyperkalemia is suspected, a repeat whole-blood sample should be submitted for immediate potassium analysis by point-of-care (blood gas) analyzer.

Summary

Pseudohyperkalemia is one of the most commonly encountered errors in laboratory testing and should be considered when there is no evidence to support a diagnosis of hyperkalemia.

Signs and symptoms which are usually evident in those with severe hyperkalemia include:

- characteristic ECG changes (e.g. peaked T waves)
- generalized weakness; muscle weakness/ muscle cramps; and paresthesia
- severe hyperkalemia can cause global paralysis.

Absence of signs and symptoms, along with no evidence of renal dysfunction (adequate urine output, normal urea/creatinine/eGFR) and no use of prescribed drugs that may increase plasma potassium, should raise suspicion of pseudohyperkalemia; particularly if the apparent hyperkalemia is severe (>6.5 mmol/L).

A marked increase in platelet count (>500 x 109/L) or white cell count (>50 x 109/L) can cause pseudohyperkalemia, so a particularly high level of pseudohyperkalemia suspicion should be afforded patients (usually suffering hematological malignant disease) with these extreme hematological findings.

It is vital for patient safety that erroneous laboratory results such as pseudohyperkalemia are identified and not treated. The majority of cases of pseudohyperkalemia are the result of bad practice during collection and handling of samples. Repeat

sampling, paying special attention to recommended procedures that avoid risk of procedure-related pseudohyperkalemia (outlined above) is all that is required in these cases.

Those rare cases in which pseudohyperkalemia persists, despite best currently recommended practice, are due to patient-specific factors, and are best elucidated by further investigation involving the measurement of potassium concurrently in plasma samples, serum samples and whole-blood samples. It may be necessary to ensure that there is absolutely no delay between blood collection and potassium measurement (whole-blood potassium measurement using a point-of-care blood gas analyzer is well suited to this need). And it may be necessary to investigate the effect of storage temperature of blood samples before potassium measurement to elucidate pseudohyperkalemia caused by inherited red cell defects.

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